REMARKS

The present amendment to the specification corrects an obvious typographical error in the series number of a provisional U.S. patent application referenced at page 9, line 9 of the application as filed. The provisional patent application was unambiguously identified as a provisional application and the filing date was provided. Thus, one of skill in the art would have understood that the provisional application filed on December 23, 1997 was USSN 60/068,634, not USSN 06/068,634 (an application bearing a series number assigned for filing dates between 1979 and 1986). The amendment to claim 59, substituting "SIGIRR" for "TIGIRR" as a modifier of the polypeptide, clarifies the nature of the claimed subject matter without altering the scope thereof. The amendment is supported throughout the application as filed (*see, e.g.,* the section of the Detailed Description of the Invention entitled "PRODUCTION OF POLYPEPTIDES AND FRAGMENTS THEREOF" at page 24, line 11, et seq.). Accordingly, the amendment does not introduce new matter.

I. Status of the Claims

Claims 34-60 remain pending in the instant application and stand rejected under 35 U.S.C. §§ 101/112 first paragraph for lack of utility/enablement. Claims 59 and 60 stand rejected under 35 U.S.C. § 112, second paragraph for asserted indefiniteness in reciting "TIGIRR."

II. The Claimed Subject Matter

The subject matter of claims 34-60 is drawn to isolated SIGIRR (Single Ig Interleukin-1 Receptor-Related) nucleic acid molecules that comprise the sequence of SEQ ID NO:1 or are related to a nucleic acid having such a sequence, recombinant vectors comprising such a molecule, host cells comprising such vectors, and methods for producing the encoded polypeptides.

III. The Outstanding Rejections

In the Office Action mailed August 24, 2004, the Examiner rejected claims 34-60 under 35 U.S.C. § 101 for asserted lack of patentable utility; the same claims were rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement predicated on the asserted lack of utility. Claims 59 and 60 were also rejected under 35 U.S.C. § 112, second paragraph, for the assertedly indefinite recitation of "TIGIRR."

IV. Rejections under 35 U.S.C. §§ 101/112, first paragraph, should be withdrawn

Applicants maintain their traversal of the rejections of all pending claims (34-60) under 35 U.S.C. §§ 101/112, first paragraph, for assertedly failing to identify a patentable utility for the claimed subject matter, and for failing to teach how to use that which assertedly had no disclosed use. Applicants maintain that an assertion of patentable utility was made in the application as filed in the form of an assertion that the claimed compositions were useful in the diagnosis/prognosis of diseases associated with genetic rearrangements at chromosome locus 11p15.5, including loss of heterozygosity (for a definition of "heterozygosity," *see* Strickberger et al., Genetics, 2d Ed., p. 117 (MacMillan Publishing Co., Inc., New York, 1976), attached as Appendix A). The use of the claimed subject matter as a marker for such diseases (a marker directly detecting genetic rearrangements at locus 11p15.5 and thereby detecting diseases associated with such rearrangements) does not depend on the function of any nucleic acid molecule or on the function of any encoded polypeptide. One of skill in the art would recognize that the use of a marker mapping to locus 11p15.5 would be useful in such prognostic/diagnostic contexts regardless of any function of such molecules other than their capacities to hybridize to their genomic counterparts as, e.g., hybridization targets.

Applicants also maintain that the application asserts a patentable use for the claimed subject matter in the form of the contribution of SIGIRR molecules to the binding of IL-1. Interleukin-1 itself was known in the art as an important cytokine mediating inflammatory and immune responses. *See, e.g.,* Rhezzi et al., Ann. First Super. Sanita 26:263-272 (1990), attached as Appendix B. The disclosure relating to SIGIRR and its role in IL-1 binding is analogous to the invention of a nucleic acid encoding a DNA ligase as

described in Example 10 of the Revised Utility Examination Guidelines, Fed. Reg. 66:1092 (January 5, 2001), a copy of which has been made of record. In particular, as attached pages 82-85 of the New England Biolabs catalog of 1996-97 (*see* Appendix C) establishes, DNA ligases did not constitute a homogeneous class, with some ligases behaving like T4 DNA ligase (use of rATP as an energy source and catalysis of phosphodiester bond linkage of DNA or RNA) and others behaving like *E. coli* DNA ligase (use of NAD as an energy source and catalysis of phosphodiester bond linkage of DNA only). Like the heterogeneous DNA ligases that each catalyzes DNA ligation, each member of the IL-1 receptor family, including SIGIRRs, contributes to the modulation of IL-1-mediated inflammatory and/or immune responses.

In view of the foregoing utilities, each disclosed in the application as filed, Applicants are entitled to a presumption of utility as a matter of law. The Examiner has not met the burden of overcoming that presumption by establishing a *prima facie* basis for lack of utility in the form of a reasonable statement, supported by evidence and/or explanation, sufficient to conclude that one of skill in the art would have been more likely than not to question, or doubt, the truth of the asserted utilities. Moreover, in view of the post-filed references made of record to corroborate the assertions of utility provided in the application as filed, it is submitted that the Examiner cannot remedy those deficiencies.

Applicants are appealing the rejection of the claims, and the Examiner's attention is directed to the Appeal Brief, incorporated herein by reference, which provides Applicants' detailed position on these issues.

The rejection of claims 34-60 under 35 U.S.C. § 112, first paragraph, for asserted lack of enablement was predicated on the absence of an asserted (or well-established) utility for the claimed subject matter. The remarks provided above establish that the claimed subject matter was supported by an assertion of patentable utility in the application as filed. Therefore, the rejection for lack of enablement is based on a flawed premise and there is no proper support for the rejection. The Examiner has not established a

prima facie basis for rejecting claims 34-60 under 35 U.S.C. § 112, first paragraph, for lack of enablement.

Applicants respectfully submit that the rejections of claims 34-60 under 35 U.S.C. §§ 101/112, first paragraph, has been overcome and should be withdrawn.

V. The Rejection Under 35 U.S.C. § 112, Second Paragraph, Should Be Withdrawn

The Examiner has maintained a rejection of claims 59 and 60 under 35 U.S.C. § 112, second paragraph, for asserted indefiniteness in reciting "TIGIRR" polypeptides in the claimed methods for producing same. In response, Applicants have amended claim 59 by substituting the term "SIGIRR" for "TIGIRR." Claim 60 depends from claim 59 and thereby benefits from the clarifying amendment to claim 59. As a consequence of the amendment, neither claim 59 nor claim 60 recites the "TIGIRR" term at issue. Applicants maintain that "TIGIRR," like "Three Immunoglobulin domain-containing Interleukin-1 Receptor-Related," is not indefinite in the context of the structural and functional requirements imposed by the rejected claims. The Examiner has not identified any other molecule that would be identified as "TIGIRR" (or as "Three Immunoglobulin domain-containing Interleukin-1 Receptor-Related") and, thus, has not identified another molecule identified by that name that also meets each of the limitations of either of the rejected claims. Accordingly, the Examiner has failed to establish a *prima facie* basis for rejecting claims 59 and 60 under 35 U.S.C. § 112, second paragraph, and the rejection should be withdrawn for that reason. To reduce the issues on appeal, however, Applicants have effectively amended claims 59 and 60 in the present amendment, without altering the scope thereof, thereby rendering moot the instant rejection.

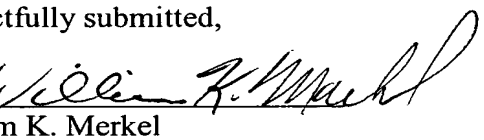
Applicants respectfully request that the Examiner exercise discretion in favor of entering the present amendment, which will reduce the issues on appeal. Upon entry of the amendment, the rejection of claims 59 and 60 under 35 U.S.C. § 112, second paragraph, will have been rendered moot and may properly be withdrawn.

VI. Conclusion

Applicants believe that all of the rejections have been overcome and/or rendered moot, and that the claims of the instant application are now in condition for allowance. Applicants request an early indication of such a favorable disposition of the case. The Examiner is invited to contact the undersigned with any questions, comments or suggestions relating to the referenced patent application.

Dated: April 26, 2005

Respectfully submitted,

By 

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GENETICS

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kled must contain factors for both. The simplest assumption, therefore is, that a hybrid plant for seed shape contains two factors, *S* and *s*. For the argument to be consistent, the pure smooth and pure wrinkled producers must also contain two factors as well, *SS* and *ss*, respectively. It is therefore clear why the *SS* and *ss* plants breed true; since they are permitted to self-fertilize, only pure lines would be expected from each type. The *Ss* hybrids, on the other hand, do not breed true, since they are each carrying both *S* and *s* factors. If we make the assumption that the *S* and *s* factors can separate or segregate from each other during gamete formation in the hybrid, then some gametes carry *S* and others *s*; i.e., there are two kinds of equally frequent pollen and two kinds of equally frequent ova. The random combinations of these gametes to form zygotes, as seen in Fig. 6–2, will then account for the observed ratios. Thus Mendel demonstrated that a hybrid between two different varieties possesses both types of parental factors, which subsequently separate or segregate in the gametes. This fundamental law is known as the *principle of segregation*, and Mendel's results have since been demonstrated many times and for many hybrid generations (Table 6–1).

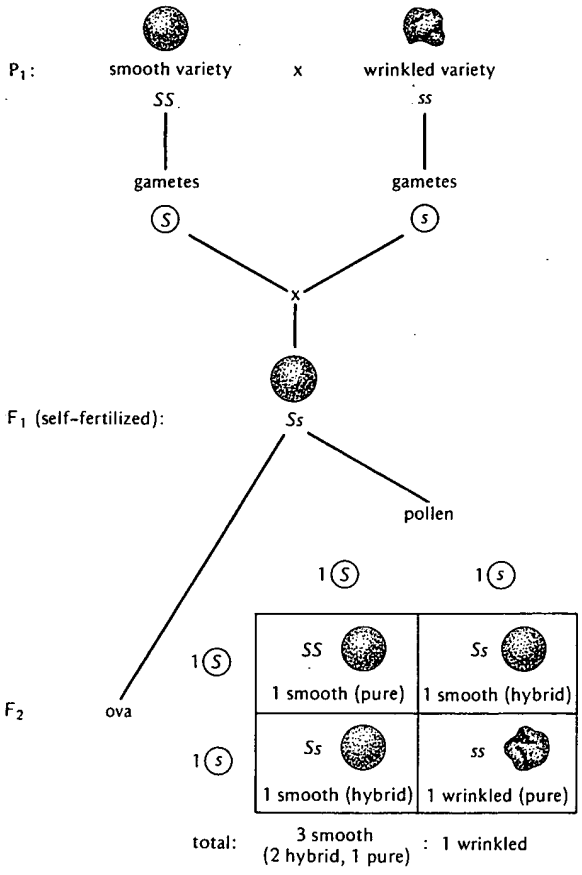

Figure 6–2

Segregation of seed-shape character and explanation for Mendel's observed results in the F_1 and F_2 generations.

TERMINOLOGY

In modern terms, an inherited factor that determines a biological characteristic of an organism is called a *gene*. In diploid organisms such as pea plants, genes exist in pairs, many of which are recognized by their production of a particular biological effect such as seed shape or pod color. The two individual genes in a particular gene pair are known as *alleles*. In some cases these alleles are identical; i.e., a wrinkled plant carries two identical alleles *s* and *s*. In some cases, such as a hybrid smooth plant, the alleles differ, one being the gene or allele for smooth, *S*, and the other being the gene or allele for wrinkled, *s*. The terms allele and gene are thus interchangeable, with the restriction that allele refers only to the genes at a particular gene pair. That is, while the gene for smooth, *S*, is an allele of (or allelic to) that for wrinkled, *s*, it is not an allele of the gene for yellow seed color, *Y*. When a gene pair in an organism contains two identical alleles, e.g., *S* and *S*, the organism is considered *homozygous* for that gene pair and is called a *homozygote*. When two different alleles are present in a single gene pair, e.g., *S* and *s*, the organism is *heterozygous* for that gene pair and is called a *heterozygote*. According to our present use of these terms, the effect of a recessive gene does not appear in a heterozygote that also contains its dominant allele. Recessive characteristics

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INTERLEUKIN 1, A PROTOTYPIC PLEIOTROPIC LYMPHOKINE

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Summary. - *Interleukin 1 (IL-1) is an endogenous mediator produced by monocytes/macrophages, endothelial cells and other cell types. It was originally identified as the main endogenous pyrogen. It was later found that IL-1 was identical to other factors that had been defined leukocytic endogenous mediator, lymphocyte activating factor. It is now clear that IL-1 has two main aspects. On one side it is an immunostimulatory molecule and on the other side is a mediator of shock and inflammation. As an immunostimulating molecule, IL-1 is a potential immunomodulating drug, while with respect to its proinflammatory action it might be important to identify antagonists or inhibitors that could be useful in the therapy of chronic inflammatory diseases.*

Riassunto (Interleuchina 1, una linfochina pleitropica prototipica). - *L'interleuchina 1 (IL-1) è un mediatore endogeno prodotto da cellule monocito/macrofagiche, endoteliali e di altro tipo. E' stato originariamente identificata come il principale pirogeno endogeno. Successivamente è stato visto che IL-1 era identica ad altri fattori che erano stati chiamati mediatore endogeno leucocitario e fattore di attivazione dei linfociti. E' ora chiaro che l'IL-1 ha due aspetti principali. Da un lato è una molecola immunostimolante e dall'altro è un mediatore dello shock e dell'infiammazione. In quanto molecola immunostimolante, l'IL-1 è un potenziale farmaco immunomodulatore, mentre considerando la sua attività proinfiammatoria sarebbe importante identificare antagonisti o inibitori che potrebbero essere utili nella terapia delle malattie infiammatorie croniche.*

Historical perspective

The term interleukin 1 (IL-1) was first proposed in 1979 [1] when it was clear that several monokine activities were actually different activities from what was then thought to be a single protein, originally designed as lymphocyte activating factor (LAF, first described by Gery and Waksman in 1974) [2]. The term IL-1 stressed the fact that LAF was a macrophage product acting on lymphocytes, and therefore acted as a communication signal between leukocytes. Thus, IL-1 was originally described as an immunoregulatory protein of interest mainly, though not only, to immunologists.

In a completely different field, researchers studying the pathogenesis of fever had focused on the role of soluble products derived from leukocytes as possible mediators of fever. In 1943, Menkins suggested that leukocytes released a pyrogenic substance and termed it "pyrexin" (for a review on the hystory of endogenous pyrogen (EP) see [3]). In 1955 Atkins and Wood [4] found a circulating pyrogenic factor in febrile rabbits that they termed endogenous pyrogen (EP). Production of EP was then demonstrated in human, murine, and rabbit mononuclear cells [3, 5]. EP was suggested to be identical to another macrophage product, known as leukocytic endogenous mediator (LEM), capable of bringing about various acute-phase changes, including hypoferremia, hypozincemia and induction of acute-phase proteins like fibrinogen [6]. Human EP was then biochemically characterized and purified to apparent homogeneity by Dinarello *et al.* in 1977 [7].

Early biochemical characterizations of LAF and EP revealed similarities between the two and in fact some reports indicated that EP and LAF might be the same protein [8, 9]. The fact that all these mediators were in fact the same became clear when, in 1984, the cloning of human IL-1β was published [10]. From then on a number of papers have reported the cloning of various IL-1 proteins from different species [11, 12].

The availability of highly purified recombinant IL-1 preparations permitted extensive characterization of its structure, biological functions and mode and the discovery of other, previously unknown, activites on a variety of cell targets.

Gene and protein structure

cDNA cloning revealed the existence of two forms of IL-1, in agreement with previous biochemical characterization of EP/LEM, which were shown to exist as two forms.

IL-1 α and β both have a molecular weight of 17 kDa but differ in their isoelectric point (pI 5.2 for IL-1α and 7.0 for IL-1β). The two forms are encoded by different genes and show very little homology. Aminoacid sequence homology between human IL-1 α and β is only 26%, while interestingly the degree of homology in the nucleic acid sequence is higher (45%) [12].

Although the extracellular, mature form of IL-1 α and β have a molecular weight of 17 kDa, the corresponding mRNAs (2.2 kB for IL-1α and 1.6 kB for IL-1β) encode translation products of about 31 kDa. While the 31 kDa IL-1α precursor is biologically active and binds the IL-1 receptor, the IL-1β precursor is inactive and does not bind the receptor [13]. These large precursor molecules are then broken down to the 17 kDa, "mature" IL-1 protein, probably by serine proteases related to plasmin and elastase [14, 15]. This processing does not seem to be a classical secretory process through the endoplasmic reticulum, and secreted IL-1 is not glycosylated, despite the presence of potential glycosylation sites. IL-1 α and β, unlike tumor necrosis factor (TNF), lack the secretory signal sequence. Unlike many secretory proteins, processing is not always required for secretion and both the 31 kDa precursor and 17 kDa mature IL-1 are secreted into the culture medium by human monocytes. A membrane-associated, biologically active 22-23 kDa form of IL-1 has also been described. After secretion, mature 17 kDa IL-1 is further adegraded and active fragments of 4.2, 4 and 2 kDa were described in human plasma from febrile patients, after exercise and in women after ovulation [16-18].

Cellular sources and regulation of IL-1 synthesis

IL-1 was originally described as a monokine, i.e. a product of activated monocytes/macrophages, that are the main sources of IL-1. Subsequent studies revealed that almost all cells produce IL-1, including T and B lymphocytes, NK cells, polymorphonuclear leukocytes, endothelial cells, fibroblasts, asynovial cells, glomerular mesangial cells, chondrocytes, astrocytes, keratinocytes, Langerhans cells and, in some cases, tumor cells [14, 15]. It is very likely that, with the availability of specific techniques for the detection of IL-1 or IL-1 mRNA, other cells will probably be found to produce IL-1.

The most popular inducers of IL-1 synthesis are bacteria and bacterial products, particularly endotoxin. Several other substances were reported to stimulate IL-1 production [19]: silica particles, phorbol esters, calcium ionophores, complement components, antigen-antibody complexes, neuropeptides like substance P, and other cytokines like TNF [20] and IL-1 itself [21]. Adherence (to plastic, endothelial cells or collagen) was also reported to induce transient expression of intracellular, membrane and secreted IL-1 by macrophages.

Biological effects of IL-1

IL-1, like the two other cytokines, TNF and IL-6, is a typical "double faced" molecule. On the one hand it is an immunomodulatory protein, acting as a communication signal between different populations of leukocytes (hence the name "interleukin", between leukocytes). The other face of IL-1 is its activity as an inflammatory mediator, acting on a wide range of cell targets. We will therefore group the different activities of IL-1 in two classes: immunomodulatory and inflammatory. In listing these activities we will also stress the cell targets of IL-1's action and in some cases we will refer to the "old" acronyms (e.g. LAF, LEM, EP) used to identify the different functions of IL-1.

IL-1 and the immune system

As indicated by the term LAF, IL-1 enhances the thymocyte mitogenic response to PHA and Con A. IL-1 was also reported to enhance the production of IL-2 by certain subsets of T cells. These two activities of IL-1 (induction of thymocyte proliferation and of IL-2 production) are at the basis of the bioassays used for the detection of IL-1 [19, 22].

The mitogenic action is not unique to IL-1 since, under appropriate experimental conditions, IL-6 and TNF are also active in the thymocyte assay [22]. Based on induction of IL-6 and on the blocking of IL-1 comitogenic action by anti IL-6 antibodies it has been proposed that the effect of IL-1 on thymocytes is indirect and is indeed mediated by induction of IL-6 by IL-1. It is of interest that in endothelial cells, unlike tymocytes, IL-1 induces IL-6 but is not active on these cells via IL-6 [23].

The role of IL-1 in induction of specific immunity, i.e. its role in antigen presentation by accessory cells, has been the subject of considerable debate [19].

IL-1 seems not to be required for activation of antigen-specific T cell clones by antigen presenting cells. In contrast, there is evidence for an important role of IL-1 in activation by antigen of virgin T cells. In the mind of the authors, an obligatory role for IL-1 in antigen presentation has not been established.

IL-1 is also active on the proliferation and differentiation of B cells. The physiological signficance of this action is not clear. More important, IL-1 could act on B cells by inducing IL-6, a lymphokine which, among other activities, has been identified as plasmocytoma/hybridoma growth factor and B cell stimulatory factor.

IL-1 has also a colony stimulating factor (CSF) activity. In fact, IL-1 accounts for the activity originally identified as hemopoietin-1 [24]. Among CSFs, IL-1 affects the earliest, most immature bone marrow precursors. In addition to having CSF activity, per se, IL-1 induces production of other CSFs, including G and GM_1 in endothelial cells [25].

The CSF/hemopoietin activity of IL-1 is a subject of considerable interest in the perspective of therapeutic application. In fact, as we will discuss later, IL-1 has

radioprotective activity and protects or restores bone marrow function compromised by the cytotoxic chemotherapeutic agents cyclophosphamide and 5 fluorouracil. The potential clinical relevance of these observations is currently being explored. Moreover, certain acute myeloid leukemias express IL-1 mRNA and protein and IL-1 could play a role in autocrine stimualtion of neoplastic elements [26, 27]. IL-1 has been reported to stimulate monocytes/macrophages to produce other cytokines. In particular, IL-1 induces the synthesis of TNF, IL-6 and IL-1 itself, also on endothelial cells [20, 21, 23, 28, 29].

These findings are not confined to *in vitro* systems. Administration of human recombinant IL-1 to rabbits causes a biphasic fever response and, during the second peak of fever, endogenous rabbit IL-1 was detected in the circulation [21]. These self-amplification mechanisms could be particularly important in the pathogenesis of chronic inflammatory diseases.

IL-1 and inflammation

Most of the pleiotropic effects of IL-1 are associated with inflammation and is now regarded as the primary mediator of the acute-phase response. It can be detected in the circulation a few hours after the onset of infection or injury (or after injection of endotoxin) and there is a growing literature reporting the presence of IL-1 in biological fluids in pathological conditions. For instance, IL-1 was detected in patients with rheumatoid arthritis [30], and in sera from human volunteers after exercise or post-ovulation [16-18]. Treatment with IL-1 has been shown to induce hemodynamic shock in rabbits [31].

Effects on the central nervous system. - The thermoregulating neurones of the hypothalamus are the targets responsible for the "oldest" action of IL-1, induction of fever. This pyrogenic activity is mediated by induction of prostaglandin synthesis, particularly PGE2. IL-1 induced prostaglandin release from hypothalamic tissue *in vitro* and its pyrogenic action is prevented by cyclooxygenase inhibitors [3]. In addition to fever, IL-1 induces slow-wave sleep [32] and anorexia [33]. All these effects can be observed upon intracerebroventricular injection of IL-1 or when IL-1 is given systemically [3, 34].

IL-1 also has important effects on the neuroendocrine system. It stimulates the release of corticotropin releasing factor (CRF) that in turn induces the release of ACTH and corticosteroids [35, 36]. Since corticosteroids are potent inhibitors of IL-1 synthesis, this could constitute an effective feedback system for controlling IL-1 synthesis *in vivo*.

The origin of IL-1 acting on the brain is still unclear. It seems unlikely that IL-1 can cross the blood-brain barrier, and although it can be produced by astrocytes, fever is also observed when IL-1 is induced by local inflammation in the periphery or is administered intraperitoneally or intravenously. The availability of techniques like immunohistochemistry or *in situ* hybridization may help answer this question, and the recent findings of IL-1 and its receptor in the brain are particularly important in this respect. In this direction, it is important to note that neuropeptides, like substance P, were reported to induce IL-1 synthesis.

Hepatic effects and the acute-phase response. - The hepatocyte is the main target for induction of the synthesis of acute-phase proteins by IL-1 [37]. These include, among other proteins, C-reactive protein, serum amyloid A, antiproteases and fibrinogen. In some cases, like serum amyloid A, induced synthesis was observed in cultured hepatocytes stimulated with IL-1 *in vitro* [38].

For other acute-phase proteins the mechanism is probably more complex. Although administration of IL-1 induces fibrinogen, it does not induce fibrinogen synthesis *in vitro* on isolated hepatocytes or on hepatoma cell lines. In fact, earlier work indicated that monocytes produced a factor (termed hepatocyte stimulating factor, HSF) that stimulated fibrinogen synthesis by hepatocytes. HSF is distinct from IL-1 and was later found to be identical to IL-6 [39, 40]. Since IL-1 is a powerful inducer of IL-6/HSF [23, 28] it seems likely that induction of some acute-phase proteins is mediated by IL-6. While the synthesis of a large number of specific acute-phase proteins, often with antitoxic properties, is typical for the IL-1-induced acute-phase response, some negative acute-phase reactants were identified. The levels of these proteins are reduced by IL-1 treatment. One such protein is albumin, whose synthesis is blocked at the transcriptional level by IL-1 [38].

Another protein decreased by IL-1 is cytochrome P-450 (in fact a family of proteins), a key protein in the oxidase system responsible for the metabolism of drugs and detoxification of foreign compounds. Treatment of mice with IL-1 or exposure of hepatocytes to IL-1 *in vitro* resulted in decreased drug metabolizing activities [41], and this effect probably explains earlier reports of reduced liver drug metabolism associated with infection and inflammation.

The overall pathway of modulation of liver functions by IL-1 is depicted in Fig. 1.

Besides inducing liver acute-phase protein synthesis, IL-1 induces other metabolic changes associated with the acute-phase response, like hypoferremia and hypozince-

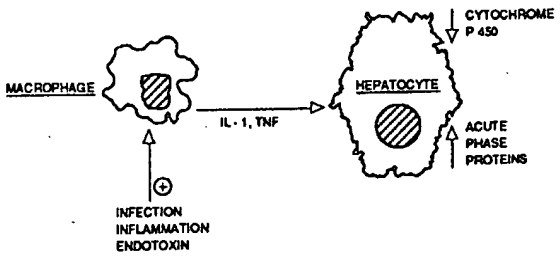

Fig. 1. - Regulation of hepatocyte functions by IL-1, IL-6 and other cytokines produced by macrophages in response to inflammatory stimuli. Cytokines act on the hepatocyte by increasing the synthesis of acute-phase proteins and decreasing that of negative acute-phase reactants, like albumin and cytochrome P 450 and related drug metabolizing enzymes.

mia [42]. Some of these changes are partially due to induction of metal-binding proteins, including methallothionein, ferritin and ceruloplasmin. Since the hypoferremic response to IL-1 is nearly abolished in neutropenic mice, it is likely that this effect is mediated via neutrophils, possibly through secretion of lactoferrin. In general, hypoferremia is considered part of what is called "nutritional immunity", since iron is an essential factor for many bacteria and its low availability limits for their growth.

IL-1 and endothelial cells. - IL-1, and the functionally related lymphokine TNF, induces a complex reprogramming of functional status of endothelial cells [43]. It was originally observed that IL-1 induces production of the arachidonate metabolite prostacycline (PGI2) and procoagulant activity (PCA) in endothelial cells [44-46]. These observations were followed by a flood of studies describing a variety of changes in endothelial cell properties after IL-1. These include production of platelet activating factor, of plasminogen activator (PA-1), of leukocyte adhesion molecules and of von Willebrand factor. Moreover, IL-1 stimulated endothelial cells produce various cytokines including IL-6, IL-8 (Sica *et al.*, unpublished) and CSFs. It is of interest that G and GM-CSF have recently been shown to modulate endothelial cell migration and proliferation [47]. All in all, the alterations in endothelial cell function induced by IL-1 cause vasodilation (via PGI2) and render the vessel wall prothrombotic by favouring the activation of the coagulation cascade (e.g. PCA) and inhibiting fibrinolysis (PA-1).

IL-1-treated endothelial cells recruit leukocytes from the blood compartment by expressing adhesion structures and producing chemotactic cytokines (IL-8). Thus, from these and other studies, endothelial cells have emerged as active strategically located compartment of inflammatory and immunological processes (Fig. 2).

Other effects of IL-1. - Some effects of IL-1 suggest it has a role in the pathogenesis of rheumatoid arthritis.

IL-1 induces proliferation of fibroblasts and synovial cells, induces collagenase secretion by synovial cells and chondrocytes, blocks proteoglycans and collagen synthesis and stimulates bone resorption by osteoclasts [48].

IL-1 induces prostaglandin synthesis and proteolysis in muscle. A factor known as PIF (proteolysis inducing factor), detected in the circulation of febrile patients was in fact found to be an active fragment of IL-1 [49].

It has also been suggested that IL-1 plays a role in the development of insulin-dependent diabetes, a pathology originating, at least partly, from autoimmune attack destroying insulin-producing β cells of the pancreas. Diabetic islets contain large numbers of macrophages, previously shown to produce substances toxic to β cells. In fact, recombinant IL-1 was toxic to β cells, suggesting it may have a part in the early stages of β cell destruction [50].

Pharmacology of the IL-1 system

In outlining the pharmacological relevance of IL-1, we must stress the fact that it can be viewed both as an immunomodulatory agent and as an inflammatory mediator. As an inflammatory mediator, IL-1 could be an important target for pharmacological intervention, with the development of antagonists or inhibitors. Although we are still far from the development of novel antinflammatory agents acting as anti-IL-1 molecules, some inhibitors have been identified that may help study the IL-1 system.

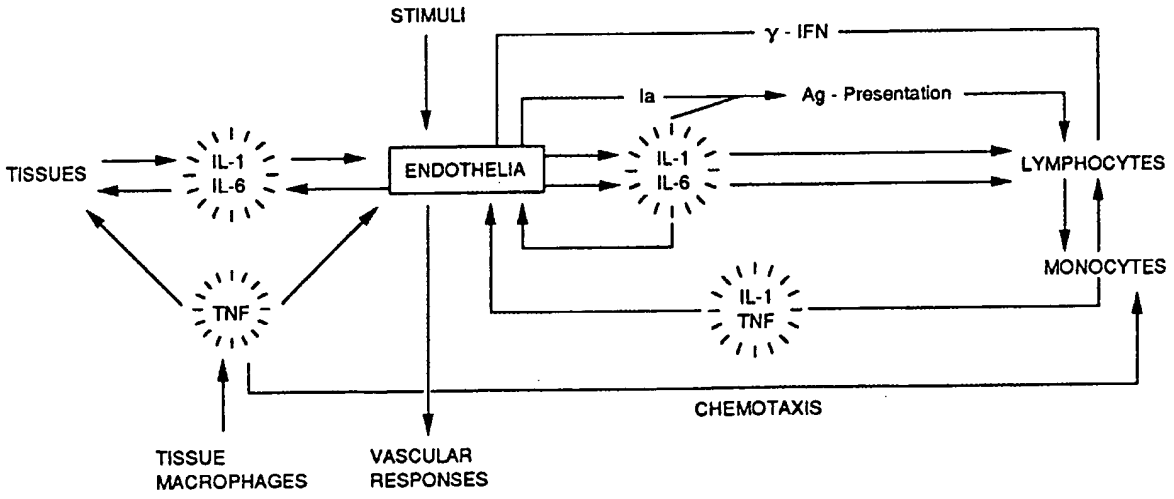

Fig. 2. - The hypothetical central role of IL-1, IL-6 and TNF in communication between endothelium and leukocytes or tissues. Endothelial cells can act as accessory cells (expression of Ia and production of IL-1 and IL-6) and initiate immune responses. These, in turn, via leukocyte IL-1, TNF and γ-IFN, can influence endothelial cell function. More in general IL-1 and IL-6 could serve as a communications signal between vessel walls and extravascular tissues. TNF, released by mononuclear phagocytes in tissue, may mediate tissue damage, alter endothelia and recruit leukocytes from the blood compartment.

IL-1 was reported to have some protective and restorative properties as an immunostimulatory agent. Many of these activities have been described *in vivo* in animal models, and in some cases clinical trials are already in progress.

Adjuvant activity

In agreement with *in vitro* data on its immunostimulatory activity IL-1 enhances *in vivo* antibody responses to protein antigens. IL-1 stimulates both the immune response to a T cell-dependent antigen, like sheep red blood cells (SRBC), but also to a T helper-independent antigen, like the poorly immunogenic polysaccharidic antigen S III from *Streptococcus pneumoniae* [51, 52].

Radioprotective and myelorestorative properties

It has long been known that immunomodulatory agents exert radioprotective effects *in vivo*. The fact that these substances, which include endotoxin, muramyl dipeptide and bacteria, also stimulate IL-1 production suggested that IL-1 could be a radioprotective agent itself.

Pretreatment of mice with recombinant IL-1 did in fact protect against the lethal effects of ionizing radiations [53]. The mechanisms by which IL-1 exerts this effect are not known.

Induction of acute-phase proteins was suggested to play a role, since some of these proteins (e.g. methallothionein and ceruloplasmin) could act as free radical scavengers. Recently IL-1 was reported to induce endogenous manganese-containing superoxide dismutase, an enzyme previously shown to have radioprotective effects [54]. The ability of IL-1 to induce granulocyte-macrophage colony stimulating factor (GM-CSF) could be important in this effect [55].

In vivo, IL-1 promotes earlier hematopoietic recovery after lethal and sublethal levels of irradiation, probably as a consequence of the stimulation of colony forming cells (CFC) into cell cycle prior to irradiation. It has in fact been suggested that CFC in S phase are more resistant to radiation than in other phases of the cell cycle [56, 57].

IL-1 restores T cell functions in mice immunosuppressed by irradiation and has myelorestorative effect in cyclophosphamide treated mice [57]. It prolongs survival if administered before or after otherwise lethal doses of cyclophosphamide, the sequence and timing of treatment being critical for this effect. These studies have prompted clinical trials to test IL-1 as a radioprotective and chemoprotective drug.

Protection from oxigen toxicity

Exposure to a hyperoxic atmosphere causes pulmonary damage and death. The only agent known to induce tolerance to hyperoxia was endotoxin. However IL-1, when administered to rats in association with TNF immediately before exposure to 99% oxygen, markedly enhanced survival and relieved pulmonary toxicity as evaluated by histological examination and hemodynamic parameters [58].

Since free radicals play an important role both in oxygen toxicity and radiation damage, it was suggested that induction of antioxidant proteins, mentioned before, might be at the basis of these protective effects of IL-1.

Induction of nonspecific resistance to infection

Several substances reportedly enhance nonspecific resistance to infections. Once again, these substance (endotoxin, bacillus Calmette-Guérin, muramyl dipeptides) are IL-1 inducers. Using recombinant IL-1 preparations, it has been demonstrated that administration of IL-1 24 h before infection prolonged survival of granulocytopenic mice lethally infected with *Pseudomonas aeruginosa*, without affecting the number of bacteria cultured from various organs [59]. The mechanism of this protective effect of IL-1 appeared related to the ability of IL-1 to down-regulate its own effects (IL-1 is a mediator of septic shock) and it was suggested that it was to some extent due to induction of "detoxifying" acute-phase proteins, particularly endotoxin-binding proteins, cytokine inhibitors and others.

Antagonists and inhibitors

The evidence that IL-1 is involved in the pathogenesis of a variety of inflammatory diseases, rheumatoid arthritis and diabetes has made this cytokine a target for possible pharmacological intervention.

One approach would be to develop drugs that block IL-1 production. To date, the best-known inhibitors of IL-1 synthesis and release are corticosteroids [60]. Prostaglandin E2 was also reported to reduce IL-1 production by elevating intracellular levels of cAMP, and this appears to be a major feedback mechanism controlling IL-1 synthesis, since IL-1 is a potent stimulus to PGE2 production [61].

Since processing IL-1 seems to require proteolytic cleavage, inhibitors of the proteases involved in IL-1 processing could stop the release of biologically active IL-1.

Another agent shown to inhibit IL-1 synthesis is interferon gamma (γ-IFN). Gamma-IFN blocks IL-1 induction by itself [62] and could therefore be important in breakin self-amplification of IL-1's effects in chronic diseases.

Gamma-IFN prevents other effects of IL-1, like induction of prostaglandin production and bone resorption. This prevention of IL-1 synthesis and action could be relevant to the pharmacological effects of γ-IFN, which is now being tested in patients with rheumatoid arthritis [63].

A recent report indicated that dietary supplementation with eicosapentenoic acid ("fish oil") reduces IL-1 production by mononuclear cells of human volunteers by 70% [64]. This finding opens a new field for manipulating IL-1 production through the diet.

A second approach to counteract IL-1's effects is to develop antagonists of its action. IL-1α and IL-1β bind to the same receptor molecule, which has recently been purified and cloned [65]. It might thus be possible to develop receptor antagonists for IL-1. The biochemical event(s) that follow IL-1/receptor interaction could also conceivably be stopped. However the second messengers of IL-1 activity are largely unknown, and in some cases receptor-independent activities of IL-1 have been proposed [66].

Since IL-1 has diverse effects on so many cell targets, that different signaling mechanisms may well be involved.

Polypeptide inhibitors of IL-1 have been identified, particularly from urine [67]. These proteins inhibit the stimulation of prostaglandin production and mitogenic activity of IL-1. Other proteins, like uromodulin, were found to bind IL-1 and therefore to inhibit its activity. In the field of peptide inhibitors, melanocyte stimulating hormone (MSH) has been reported to block IL-1 activity *in vitro* and *in vivo*.

IL-1 peptides

From the therapeutic point of view, it would be desirable to have molecules that retain IL-1's immunostimulatory properties but not its proinflammatory action. One way to dissociate these two activities was to identify the parts of the IL-1 molecule responsible for its pharmacological activity. Short fragments of IL-1 have been synthesized on the basis of their predicted exposure on the molecule surface. One of these, the highly hydrophilic fragment 163-171 of human IL-1β, has many of the *in vivo* immunomodulatory and protective properties of IL-1, including adjuvant action, restoration of immune reactivity in immunocompromised animals and radioprotection [52, 68]. However, this nonapeptide, even at high doses, did not reproduce many of the inflammatory activities of IL-1 such as fever, hypoferremia, induction of acute-phase proteins and shock [68]. These findings suggest that the region of the IL-1 molecule responsible for its immunostimulatory activity is distinct from that with inflammatory activity and that the two effects can be dissociated.

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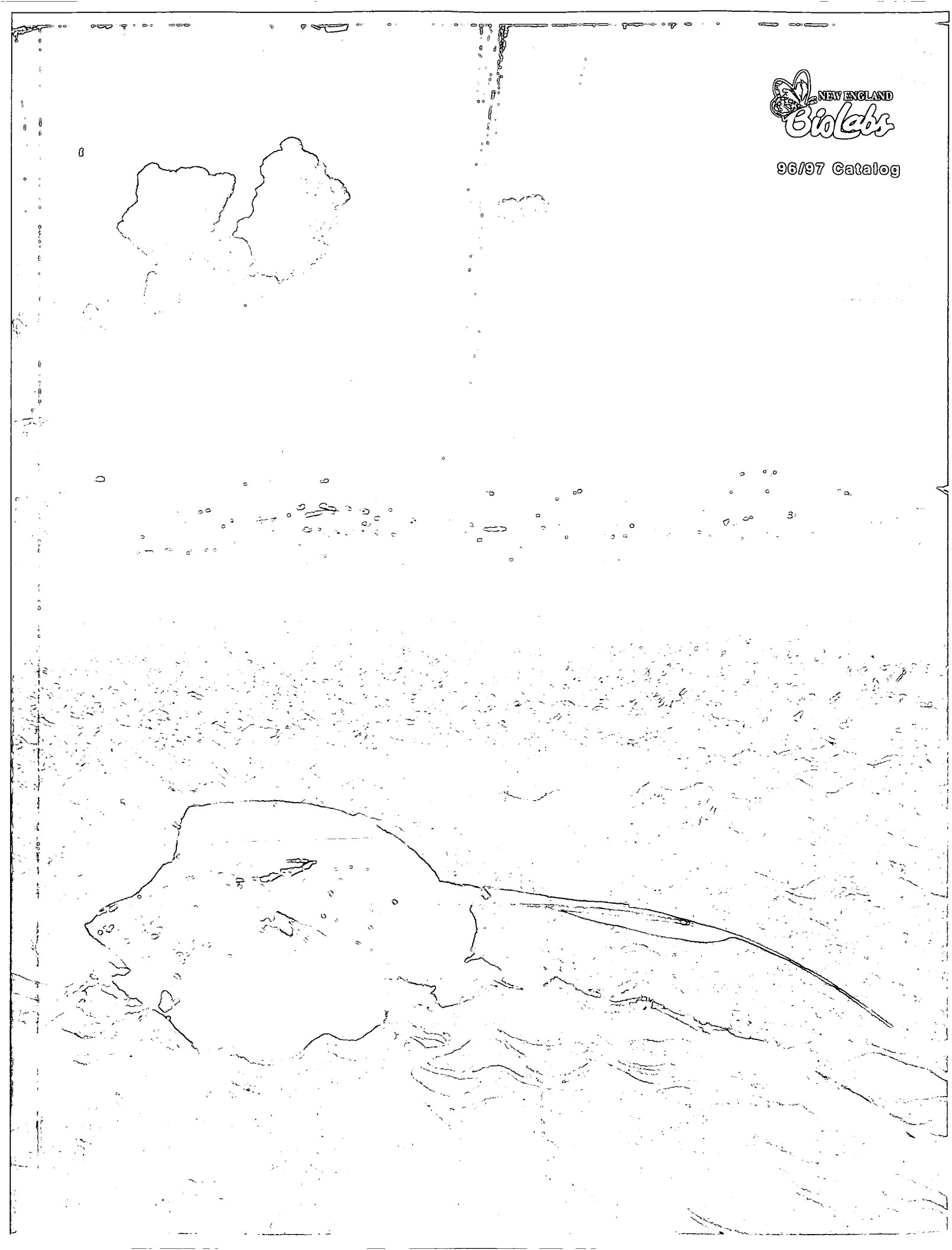
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Description: Catalyzes the formation of a phosphodiester bond between juxtaposed 5′ phosphate and 3′ hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt-end and cohesive-end termini as well as repair single stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids (1).

Source: Purified from *E. coli* C600 pcI857 pPLc28 lig8 (2).

Applications:
- Cloning of restriction fragments (3)
- Joining RNA single strands via bridging oligonucleotide adapters (4)

Reaction Buffer: 1X T4 DNA Ligase Buffer: [50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 25 µg/ml bovine serum albumin]. Recommended DNA concentration (0.1 to 1 µM in 5′ termini). **Optimal ligation occurs at 16°C.**

Unit Definition: One NEB unit is defined as the amount of enzyme required to give 50% ligation of *Hind* III fragments of lambda DNA in 30 minutes at 16°C in 20 µl of the above assay mixture and a 5′ DNA termini concentration of 0.12 µM (300 µg/ml).

One cohesive end ligation unit equals 0.015 Weiss (ATP-PP exchange) units (5). Equivalently, one Weiss unit equals 67 cohesive-end ligation units.

Heat Inactivation: T4 DNA Ligase can be inactivated by incubation at 65°C for 10 minutes.

Notes on Use: *Cohesive End Ligation:* For most cohesive end ligations, a 30 minute incubation at 20°C is often sufficient (see figure 1). Generally, incubations are at 16°C for 4-16 hours.

Blunt End and Single-base Overhang Ligations: Ligation of blunt-ended and single-base pair overhang fragments requires about 50 times as much enzyme to achieve the same extent of ligation as cohesive-end DNA fragments. Despite this fact, the concentration of ligase offered by New England Biolabs is more than adequate to achieve greater than 95% ligation of blunt-ended DNA fragments in a short period of time (see figure 2).

Blunt-end ligation may be enhanced by addition of PEG (6) or hexamine chloride (7), or by reducing the rATP concentration to 50 µM (8).

ATP is an essential cofactor for the reaction. This contrasts with *E. coli* DNA ligase which requires NAD.

To dilute T4 DNA ligase that will subsequently be stored at –20°C, 50% glycerol storage buffer (Diluent Buffer A, #008-A) should be used; to dilute for immediate use, 1X T4 DNA Ligase reaction buffer can be used.

Ligation can also be performed in all of the four restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer if they are supplemented with 1 mM ATP.

Quality Assurance: Purified free of contaminating endonucleases and exonucleases. Each lot of T4 DNA ligase is also tested in a mock cloning assay which reveals any damage to the ligated DNA termini. Greater than 99.9% of the termini remain undamaged in this assay.

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml bovine serum albumin and 50% glycerol.
Store at -20°C.

Concentration: 400,000 and 2,000,000 units/ml.

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(5) Weiss, B., et al. (1968) *J. Biol. Chem.* 243, 4543–4555.
(6) Upcroft, P. (1987) *Gene* 51, 69–75.
(7) Rusche, J. (1985) *Nucl. Acids Res.* 13, 1997-2008.
(8) New England Biolabs, unpublished observations

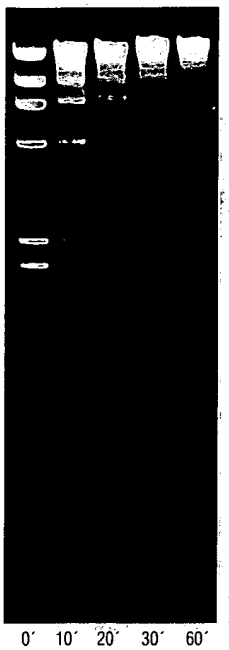


Figure 1: Ligation of Hind III fragments of lambda DNA using 1 unit (1 µl of a 1:400 dilution) of T4 DNA ligase.

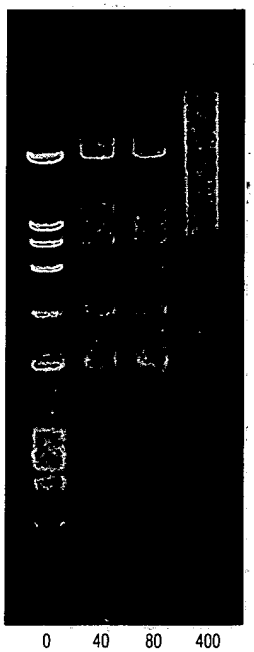


Figure 2: Ligation of blunt-ended Hae III fragments of lambda DNA using various amounts of T4 DNA ligase in a 20 µl reaction volume. Incubated for 30 minutes at 16°C.

Cloned at NEBiolabs RR Recombinant Enzyme

RR *Thermus aquaticus* (*Taq*) DNA Ligase

Description: *Taq* DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3´ hydroxyl termini of two adjacent oligonucleotides which are hybridized to a complementary target DNA. The ligation will occur only if the oligonucleotides are perfectly paired to the complementary target DNA and have no gaps between them; therefore, a single-base substitution can be detected. *Taq* DNA Ligase is active at elevated temperatures (45°C–65°C) (1,2).

Source: Purified from an *E. coli* strain containing the cloned ligase gene from *Thermus aquaticus* HB8 (1).

Applications:
- Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction (1,3).
- Mutagenesis by incorporation of a phosphorylated oligonucleotide during PCR amplification (4).

Reaction Buffer: 1X *Taq* DNA Ligase Buffer: [20 mM Tris-HCl (pH 7.6 @ 25°C), 25 mM Potassium Acetate, 10 mM Magnesium Acetate, 10 mM DTT, and 1 mM NAD, and 0.1% Triton X-100]. Incubate at 45°C.

Notes on Use: A cohesive end unit is equivalent to the nick-closing unit (1).

Requires NAD^+ as a cofactor. NAD^+ is supplied in the 10X *Taq* Ligase Buffer; the buffer should be kept frozen to extend the half life of the NAD^+ cofactor.

Quality Assurance: Each lot is tested for contaminating single-stranded DNA exonuclease, endonuclease, ribonuclease and phosphatase activities.

Unit Definition: (Cohesive End Unit)
One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of *Bst*E II-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C.

Unit Assay Conditions: 1X *Taq* DNA Ligase Buffer and DNA (20 µg/ml). After incubation at 45°C for 15 minutes, the reaction is terminated by addition of stop dye (50% glycerol, 50 mM EDTA and bromophenol blue), heated at 70°C for 10 minutes and then loaded on a 0.7% agarose gel.

Concentration: 40,000 units/ml.

Storage Buffer: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1.0 mM dithiothreitol, 200 µg/ml bovine serum albumin and 50% glycerol. Store at –20°C.

Note: *Taq* DNA Ligase should not be used as a substitute for other DNA ligases, i.e.,T4 DNA Ligase.

References:

(1) Barany, F. (1991) *Proc. Natl. Acad. Sci. USA* 88, 189.
(2) Takahashi, M. et al. (1984) *J. Biol. Chem.* 259, 10041–10047.
(3) Barany, F. (1991) *The Ligase Chain Reaction in a PCR World*, Cold Spring Harbor Laboratory Press ISSN pp. 5–16.
(4) Michael, S.F. (1994) *Biotechniques* 16, 411–412.

#208S	2,000 units	**$60**
#208L	10,000 units	**$240**

- *Isolated from a recombinant source*
- *Thermostable Ligase for Incorporation of Phosphorylated Oligonucleotides during PCR and Ligase Chain Reaction*
- *Taq DNA Ligase is NOT a Substitute for T4 DNA Ligase*
- *Supplied with 10X Reaction Buffer containing NAD^+*

T4 RNA Ligase

#204S 1,000 units **$44**
#204L 5,000 units **$176**

- *Isolated from a recombinant source*
- *Ligation of single-stranded RNA and DNA*
- *Labeling 3´-termini of RNA with 5´-[^{32}P] pCp*
- *Synthesis of Single-stranded Oligonucleotides*
- *Supplied with 10X Reaction Buffer*

Description: Catalyzes ligation of a 5´ phosphoryl-terminated nucleic acid donor to a 3´ hydroxyl-terminated nucleic acid acceptor through the formation of a 3´→5´ phosphodiester bond, with hydrolysis of ATP to AMP and PP_i. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates (1)

Source: Purified from *E. coli* strain RRI containing the plasmid pRF-E35 [constructed at New England Biolabs, Inc. after the method of K.N. Rand and M.J. Gait (2)].

Applications:
- Labeling of 3´-termini of RNA with 5´-[^{32}P] pCp (3)
- Inter- and intramolecular joining of RNA and DNA molecules (4,5)
- Synthesis of single-stranded oligodeoxyribonucleotides (6)
- Incorporation of unnatural amino acids into proteins (7)

Reaction Buffer: 1X RNA Ligase Buffer: [50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM dithiothreitol, and 1 mM ATP]. Incubate at 37°C.

Notes on Use: Heat inactivated by incubating at 65°C for 15 minutes or boiling for 2 minutes.

Addition of DMSO to 10% (v/v) is required for pCp ligation (3)

Quality Assurance: Each lot is tested for contaminating single-stranded DNA exonuclease, endonuclease, ribonuclease and phosphatase activities.

Unit Definition: One unit is defined as the amount of enzyme required to convert 1 nmole of 5´-phosphoryl termini in 5´-[^{32}P]rA_{20} to a phosphatase-resistant form in 30 minutes at 37°C (1).

Unit Assay Conditions: 1X RNA Ligase NEBuffer and RNA (10 µM 5´-[^{32}P]rA_{20}, 10 µM in 5´ termini). After incubation at 37°C for 15 minutes, the reaction is terminated by boiling for 2 minutes, and the bacterial alkaline phosphatase-resistant 5´ phosphoryl termini are determined as described (8).

Concentration: 20,000 units/ml.

Storage Buffer: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, and 50% glycerol. Store at –20°C.

References:

(1) England, T., Gumport, R. and Uhlenbeck, O. (1977) *Proc. Natl. Acad. Sci. USA* 74, 4839-4842.
(2) Rand, K.N. and Gait, M.J. (1984) *EMBO J.* 3, 397-402.
(3) England, T. and Uhlenbeck, O. (1978) *Nature* 275, 560-562.
(4) Romaniuk, P. and Uhlenbeck, O. (1983) *Methods Enzymol.* 100, 52-56.
(5) Moore, M.J. and Sharp, P.A. (1992) *Science* 256, 992-997.
(6) Tessier, D.C., Brousseau, R. and Vernet, T. (1986) *Anal. Biochem.* 158, 171-178.
(7) Noren, C.J. et al. (1989) *Science* 244, 182-188.
(8) Sugino, A. et al. (1977) *J. Biol. Chem.* 252, 3987-3994.

DNA Ligase (*E. coli*, NAD)

#205S 200 units **$44**
#205L 1,000 units **$176**

- *Specific Activity: 6,000 units/mg*
- *Supplied with 10X Reaction Buffer*

Description: Catalyzes the formation of a phosphodiester bond between juxtaposed 5´-phosphate and 3´-hydroxyl termini in duplex DNA containing cohesive ends.

Source: Purified from *E. coli* strain 594 (su$^-$) carrying the prophage λgt4 *lop*11 *lig·Sam* 7 (1) by the procedure of Panasenko et al. (2).

Applications:
- Okayama and Berg cDNA cloning (3)

Reaction Buffer: 1X *E. coli* DNA Ligase Buffer: [50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM dithiothreitol, 26 µM NAD^+, 25 µg/ml bovine serum albumin]. **Optimal ligation occurs at 16°C.**

Notes on Use:
Requires NAD^+ (nicotinamide adenine dinucleotide) as a cofactor, in contrast to other ligases which use rATP.

Ligation of blunt ended fragments is extremely inefficient. For ligation of blunt ended fragments use T4 DNA ligase.

Does not ligate RNA to DNA (4).

Quality Assurance: Free of contaminating endonucleases and exonucleases.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of *Hind* III fragments of lambda DNA in 30 minutes at 16°C in 20 µl of the assay mixture below and a 5´ DNA termini concentration of 0.12 µM (300 µg/ml).

Unit Assay Conditions: 30 mM Tris-HCl (pH 8.0), 4 mM $MgCl_2$, 1 mM dithiothreitol, 26 µM NAD^+, 50 µg/ml bovine serum albumin and *Hind* III fragments of lambda DNA (300 µg/ml).

Concentration: 4,000 units/ml.

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml bovine serum albumin and 50% glycerol.
Store at –20°C.

References:

(1) Panasenko, S.M. et al. (1977) *Science* 196, 188–189.
(2) Panasenko, S.M. et al. (1978) *J. Biol. Chem.* 253, 4590–4592.
(3) Okayama, H. and Berg, P. (1982) *Mol. Cell. Biol.* 2, 161-170.
(4) Higgins, N.P. and Cozzarelli, N.R. (1979) *Methods Enzymol.* 68, 50-71.

Nuclease BAL-31

Description: BAL-31 exonuclease degrades both 3′ and 5′ termini of duplex DNA without generating internal scissions. The enzyme is also a highly specific single-stranded endonuclease which cleaves at nicks, gaps and single-stranded regions of duplex DNA and RNA (1,2).

Source: Purified from the culture medium of *Alteromonas espejiana* BAL-31. Contains a mixture of "fast" and "slow" species of the enzyme (3).

Applications:
- Progressive shortening of double-stranded DNA fragments at both termini (4)
- Restriction site mapping (2).

Reaction Buffer: 1X Nuclease BAL-31 Buffer: [600 mM NaCl, 12 mM $CaCl_2$, 12 mM $MgCl_2$, 20 mM Tris-HCl (pH 8.0), 1 mM EDTA]. **Incubate at 30°C.**

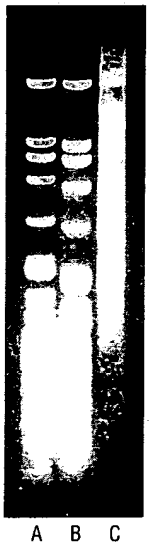
Notes on Use:
Heat inactivated by incubation at 65°C for 10 minutes in the presence of 20 mM EGTA, a specific chelator of the essential cofactor Ca^{2+}. This treatment does not affect the Mg^{2+} concentration.

Duplex products of the exonuclease are a mixture of blunt and staggered ends. This mixture can be cloned directly, although maximal ligation efficiency requires repairing the staggered ends with a suitable DNA polymerase.

If necessary, the enzyme may be diluted in reaction buffer just prior to use.

Activity is linear with enzyme concentration.

Ligation of Nuclease BAL-31 treated fragments



A B C

A) Gel electrophoresis of lambda DNA-Hae III digest

B) lambda DNA-Hae III digest after 2 minute incubation with one unit of BAL-31

C) As in (B) followed by incubation with T4 DNA ligase

Quality Assurance: Purified free of detectable double-stranded endonuclease activity.

Unit Definition: One unit is defined as the amount of enzyme required to remove 200 base pairs from each end of linearized double-stranded φX174 DNA (650 µg/ml) in 10 minutes at 30°C in a total reaction volume of 50 µl using the above reaction conditions.

Concentration: 1,000 units/ml.

Storage Buffer: 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 0.25 mM EDTA, 200 µg/ml bovine serum albumin and 50% glycerol. Store at –20°C.

References:

(1) Gray, H.B. et al. (1975) *Nucl. Acids Res.* 2, 1459–1492.
(2) Legerski, R.J. et al. (1978) *Nucl. Acids Res.* 5, 1445–1463.
(3) Wei, C.-F. et al. (1983) *J. Biol. Chem.* 258, 13506-13512.
(4) Sambrook, J. et al. (1989) *Molecular Cloning: A Laboratory Manual*, second edition, pp. 5.73-5.75, Cold Spring Harbor Laboratory, Cold Spring Harbor.

#213S	50 units	**$44**
#213L	250 units	**$176**

- *Progressive shortening of duplex DNA*
- *Inactivation by treatment with EGTA*
- *Supplied with 2X Reaction Buffer*